Data Evaluation Report on the Sub-acute Toxicity of Stabilan to Rainbow Trout (*Salmo gairdneri*)

PMRA Submission Number {.....} EPA MRID Number 467152-18

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D325185
	OECD Data Point	{.....}
	EPA MRID	467152-18
	EPA Guideline	Non-guideline (OECD 204)

Test material: Stabilan **Purity:** 465 g ai/L
Common name Chlormequat chloride
Chemical name: IUPAC: 2-Chloroethyltrimethyl ammonium chloride
CAS name: 2-Chloro-N,N,N-trimethylethanammoniumchloride salt
CAS No.: 999-81-5
Synonyms: Chlorocholine chloride

Primary Reviewer: Brian D. Kiernan
EPA/OPP/EFED/ERBIV

Date: 6/21/2006
10/17/2006

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	018101	

Date Evaluation Completed:

CITATION: Bogers, M. 1991. 21-day prolonged toxicity study with Stabilan in the rainbow trout. Unpublished study performed by RCC Notox B.V., Hertogenbosch, The Netherlands. Laboratory Report No. 1250/FP9 (001046). BASF Registration Document No. 1991/1002363. Study submitted by BASF Corporation, Research Triangle Park, NC. Study initiated February 1, 1989 and submitted April 15, 1991.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the sub-acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 21-day sub-acute toxicity study, rainbow trout (*Salmo gairdneri*) were exposed to Stabilan (containing 465 g chlormequat chloride/L) under static renewal conditions at nominal concentrations of 0 (negative control) and 100 mg product/L (reported limit test). The purity of the test material was reported to be 465 g ai/L; the density of the product was not provided, so % purity could not be calculated. The TWA measured concentration was 78.5 mg product/L and excessive analytical variability was observed in day 0 (67-94% of nominal) and corresponding day 2 (62-90% of nominal) recoveries.

No treatment-related effects on mortality, clinical signs of toxicity, or terminal wet weight or length were observed. However, control mortality was 60% in one of two replicate vessels by study termination (overall average of 30%). The 21-day LC_{50} was >78.5 mg product/L, and the NOAEC for all endpoints was 78.5 mg product/L.

Currently, there is no U.S. EPA requirement or guidance for a sub-acute freshwater fish toxicity study. However, due to unexplained control mortality and excessive analytical variability in day 0 samples, this study is not scientifically sound and does not provide useful information on the 21-day sub-acute toxicity of Stabilan to the rainbow trout (*Salmo gairdneri*). This toxicity study is classified as UNACCEPTABLE.

Results Synopsis

Test Organism Size/Age (mean weight or length): Age not reported; mean of 1.08 g and 4.4 cm
Test Type (Flow-through, Static, Static Renewal): Static renewal

21-Day Survival:
LC_{50}: >78.5 mg product/L 95% C.I.: N/A
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Clinical signs of toxicity
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Wet Weight
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Length
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OECD Guidelines for Testing of Chemicals, No. 204 (1984). General deviations from OPPTS guidance involving acute (850.1075) and/or early life stage (850. 1400) toxicity studies with rainbow trout included:

1. The % active ingredient of chlormequat chloride in the formulated product was not reported. In addition, the storage conditions of the test material were not reported.

2. Although not recommended, tap water is permitted provided it does not affect survival or health of the test fish.

3. The pH levels (8.0-8.6) exceeded the recommended range (>6.0 and <8.0) for freshwater fish (acute toxicity studies). Values were generally within acceptable OECD limits.

4. The test temperature of 14.5-16.5 °C was slightly higher than the 12 ± 2.0 °C temperature recommended for rainbow trout. Values were within acceptable OECD limits.

5. In this study, one of the two control replicates had a mortality rate of 60% (3/5) by day 21. All mortality occurred between days 15 and 21. Control mortality cannot exceed 10% in acute toxicity studies.

6. Recoveries of the test substance were variable in freshly-prepared solutions on day 0, exceeding 20% of the mean value of 76.6% (range of 67.4-94.5%). Recoveries obtained in freshly-prepared solutions on day 20 were more precise, ranging from 84.0-103.0% of nominal (mean of 92.6%). Measured concentrations were only determined on days 0 (new), 2 (old), and 20 (new).

7. The quantity of food offered each day (% of bw per fish) should have been reported.

Excessive control mortality and analytical variability on day 0 affects the scientific soundness of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material	Stabilan
Description:	Liquid
Lot No./Batch No. :	2859969
Purity:	465 g ai/L (not provided in terms of % purity)

Stability of compound under test conditions: Based on the limited data provided, Stabilan appeared to be stable under the 48-hour static renewal periods of this study. Stability was assessed in all replicates on days 0 (new) and 2 (old). Two of the day-2 samples were broken during shipment. However, in the remaining four samples, recoveries were 92-101% of day-0 values in corresponding replicates (reviewer-calculated).

(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Physicochemical properties of Chlormequat Chloride.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

Storage conditions of Test chemicals: Not reported

2. Test organism:

Species: Rainbow trout (*Salmo gairdneri,* Teleostei, Salmonidae)
Age at test initiation: Not reported
Weight at study initiation: 1.08 g
Length at study initiation: 4.4 cm
Source: Parkwood Trout Farm, Harrietsham, UK

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study – Concentrations for use in the definitive study were selected based on an acute LC_{50} of >1000 mg/L (RCC NOTOX Project No. 009214/RCC Project 224526).

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 Holding: ≥14 days Acclimation: ≥7 days following a 48-hour settling in period Holding: fish were maintained under flow-through conditions in aerated tap water Acclimation: in dilution water (not further specified) Trouvit 00 once daily <5% during 7 days prior to testing	Temperature and illumination were similar during acclimation and testing. *Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Duration of the test	21 days	
Test condition Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	 Static renewal Every 48 hours	 *A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	None reported	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass vessels 10 L 10 L	*Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Filtered, aerated tap water	The tap water was not chlorinated and therefore de-chlorination was not necessary. Although not recommended, tap water is permitted provided it does not affect survival or the test fish. In this study, one of the two control replicates resulted in a mortality rate of 60% (3/5). As no other mortality was observed in any test or control replicate during the study, the water quality did not seem to be the cause of the mortality in this replicate. Periodic analysis of the dilution water for heavy metals and PAH's was provided (November 1988). *Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency=s 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf)* *Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	2.33 mmol/L (from dilution water analysis) 8.0-8.6 6.9-10.5 mg/L (approx. equivalent to ≥70% air saturation) Not determined Not determined Not determined Not determined Not determined 14.5-16.5°C N/A pH and DO were at test initiation, in old and new medium at each renewal period, and at study termination. Temperature was measured daily in a control vessel.	pH and temperature were maintained higher than recommended, but were generally within OECD recommendations. *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ∃ 60% during first 48 hrs and ∃ 40% during second 48 hrs; flow-through: ∃ 60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 2 N/A 6	 *Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 5 N/A 5	 *Number of organisms per replicate should be ∃ 10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.54 g/L (initial instantaneous)	*Recommended static conditions are # 0.8 g/L at # 17EC and # 0.5 g/L at > 17EC. Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: nominal product: measured product:	0 (negative control) and 100 mg product/L <33.1 (<LOQ, control) and 91.4 mg product/L	The concentration of the test article was determined in each replicate vessel at the beginning and end of the first renewal (days 0 and 2), and in freshly prepared medium on day 20. Recoveries were highly variable in freshly-prepared solutions on day 0, ranging from 67.4-94.5% of nominal (mean of 76.6%). Although variable among replicates on day 0, data obtained on day 2 indicated that the test solutions were stable during the 48-hour static period (similar recoveries in corresponding replicates). Recoveries obtained on day 20 were more precise, ranging from 84.0-103.0% of nominal (mean of 92.6%). The mean-measured concentration was a reviewer-calculated TWA.
Solvent (type, percentage, if used)	N/A	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	14 hours light/8 hours dark	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*

Parameter	Details	Remarks *Criteria*
Feeding	Fish were fed daily with Trouvit 00; quantity not reported.	*Fish should not feed during the study.*
Recovery of chemical: Frequency of determination: Level of quantization Level of detection	Mean of 85% of nominal Days 0 and 20 33.1 mg product/L Not reported	From freshly-prepared solutions.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects/toxicity symptoms	- Mortality - Clinical signs of toxicity - Body weight - Body length	
Observation intervals	The fish were observed daily for mortality and clinical signs of toxicity. Mean body weights and lengths were determined at study initiation and termination.	*Observation intervals should be a minimum of every 24 hours.*
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Cumulative mortality was 60% (3/5) in one of the two control replicates. No other mortality occurred in any control or treatment replicate. The 21-day LC_{50} was therefore estimated to be >100 mg product/L, and the NOAEC for mortality was 100 mg product/L.

Table 3: Effect of Stabilan on Mortality of Rainbow Trout.

Treatment mg product/L Mean-measured (and nominal)	No. of fish at start of study	Observation period					
		Day 7		Day 14		Day 21	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control Rep A	5	0	0	0	0	3	60
Negative control Rep B	5	0	0	0	0	0	0
78.5 (100)	30	0	0	0	0	0	0
NOAEC	100 mg product/L						
LC_{50}	>100 mg product/L						
Positive control, if used mortality: LC_{50}:	N/A						

B. NON-LETHAL TOXICITY ENDPOINTS:

No clinical signs of toxicity were observed during the study. The NOAEC for clinical signs of toxicity was 100 mg product/L.

Surviving fish from Replicate A (mean of 1.14 g and 5.0 cm) were notably lighter and smaller than those in control Replicate B (mean of 2.79 g and 6.1 cm). Excluding Replicate A data, no treatment-related effect on body weight or length was observed. The NOAEC for growth endpoints was 100 mg product/L.

Table 4: Effect of Stabilan on Growth of Rainbow Trout.

Treatment mg product/L Mean-measured (and nominal)	Length, cm	Wet Weight, g
Day 21		
Negative control Rep A	5.0	1.14
Negative control Rep B	6.1	2.79
78.5 (100) Rep A	6.3	2.98
78.5 (100) Rep B	5.5	2.17
78.5 (100) Rep C	6.0	2.63
78.5 (100) Rep D	6.0	2.46
78.5 (100) Rep E	6.0	2.60
78.5 (100) Rep F	5.8	2.35
NOAEC	100 mg product/L	100 mg product/L

C. REPORTED STATISTICS:

As no treatment-related mortality occurred, the 21-day LC_{50} was visually estimated. The NOAEC values for mortality, clinical signs of toxicity, and terminal wet weight were visually determined. The NOAEC for terminal length data was determined using Dunnett's multiple comparison test; each mean replicate value was compared to the negative control Replicate B (Replicate A data were excluded). Results were reported in terms of the nominal concentration (mg product/L).

21-Day Survival:
LC_{50}: >100 mg product/L 95% C.I.: N/A
NOAEC: 100 mg product/L
LOAEC: >100 mg product/L

Clinical signs of toxicity
NOAEC: 100 mg product/L
LOAEC: >100 mg product/L

Wet Weight
NOAEC: 100 mg product/L
LOAEC: >100 mg product/L

Length
NOAEC: 100 mg product/L
LOAEC: >100 mg product/L

Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Statistical analyses were not conducted because one of the control replicates provided questionable data, leaving only one replicate for the control group. The NOAEC values represent the reviewer's visual determinations and are based on the time-weighted average concentration.

21-Day Survival:
LC_{50}: >78.5 mg product/L 95% C.I.: N/A
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Clinical signs of toxicity
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Wet Weight
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Length
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Endpoints affected: None

E. STUDY DEFICIENCIES:

Currently, there is no U.S. EPA requirement or guidance for a sub-acute (21 day) freshwater fish toxicity study. Regardless, this study is not scientifically sound, and does not provide useful information on the sub-acute toxicity of Stabilan to rainbow trout (*Salmo gairdneri*) because control mortality exceeded 10%, and because highly variable analytical results were observed in freshly-prepared media on day 0 (exceeding 20% of the mean).

F. REVIEWER=S COMMENTS:

In this study, one of the two control replicates (Replicate A) had a mortality rate of 60% (3/5), resulting in an overall control mortality rate of 30% (3/10). As no other mortality was observed in any test or control replicate during the study, the water quality did not seem to be the cause of the mortality in this replicate. In addition, the two surviving fish from control Replicate A were notably smaller and weighed less than those present in Replicate B. This study author reported that the possibility of an exchange of control Replicate A with any of the test vessels containing 100 mg/L can be excluded. The study author concluded that since no other mortality or other effects were recorded in any of the remaining vessels, this indicates that all remaining fish were in good health and the results of the study are considered valid.

The concentration of the test article was determined in each replicate vessel at the beginning and end of the first renewal (days 0 and 2), and in freshly prepared medium on day 20. Recoveries were highly variable in freshly-prepared solutions on day 0, exceeding 20% of the mean recovery; recoveries were 67.4, 67.8, 68.9, 68.0, 94.5, and 93.1% for the six replicates (mean of 76.6%). On day 2, two of the six samples were broken in shipment, but analysis of the remaining four supported recoveries obtained on day 0 and indicated stability during the 48-hour static period (92-101% of day-0 values, reviewer-calculated). However, since recoveries on day 2 were similar to day 0, slightly excessive analytical variability was also observed on day 2 (mean of 72.4%, range of 62.2-89.9%). Recoveries obtained on day 20 were more precise and accurate, ranging from 84.0-103.0% of nominal (mean of 92.6%). The study author reported that since one unique procedure for the preparation of test solutions was followed during the whole test period, no explanation can be given for the relatively low values for actual concentration in part of the vessels on day 0. Since analyses were performed at a much later date than sampling, additional analyses could not be performed to assure accuracy of preparation of test concentrations at other points in time.

It was reported that no effects were recorded at 1000 mg/L (limit test) in the acute fish test that was performed before the 21-day fish toxicity test. Hence, the 21-day fish toxicity test became a limit test with 30 fish exposed to 100 mg/L Stabilan.

The prepared test solutions were clear without precipitation.

In-life dates were February 1-22, 1989.

G. CONCLUSIONS:

This study is scientifically not sound because unexplained control mortality and low recoveries in freshly-prepared day-0 solutions were observed. Although this study was not designed to fulfill any current U.S. EPA FIFRA guideline, data obtained from this study are not useful, and this study is therefore classified as UNACCEPTABLE.

21-Day Survival:
LC_{50}: >78.5 mg product/L 95% C.I.: N/A
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Clinical signs of toxicity
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Wet Weight
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Length
NOAEC: 78.5 mg product/L
LOAEC: >78.5 mg product/L

Endpoints affected: None

III. REFERENCES:

A reference list was not provided.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

	concentration	time	CT		concentration	time
100 mg/L	67.4	1	67.43	100 mg/L	67.4	1
	67.8	1	67.78		67.8	1
	68.9	1	68.92		68.9	1
	68.0	1	67.98		68.0	1
	94.5	1	94.49		94.5	1
	93.1	1	93.14		93.1	1
	62.2	3	186.6		84.0	1
	69.9	3	209.7		88.8	1
	67.6	3	202.8		91.5	1
	89.9	3	269.7		90.4	1
	84.0	1	84		98.2	1
	88.8	1	88.8		103.0	1
	91.5	1	91.5			
	90.4	1	90.4		**1015.6**	
	98.2	1	98.2			
	103.0	1	103			
		24	**1884.44**			

TWA= 78.518

Recovery = 84.637
(freshly-prepared soln's only)